Platform AG: Imaging & Optical Microscopy: Instrument and Probes Developments

1921-Plat
Dynamic Object Tracking (DOT) Microscopy To Image Endosomal Transport On Individual Microtubules In An Axon
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Fluorescence imaging with single molecule resolution has been vital to many important discoveries in biology. The spatial resolution of fluorescence microscopy has a physical lower bound, the diffraction of light, which is approximately half of the wavelength and in the range of 200-300nm for visible light. Many biological objects of interest are on a length scale below this diffraction limit. Several high resolution techniques have been developed to break this diffraction limit. Here we describe a new high-resolution imaging technique called Dynamic Object Tracking (DOT) microscopy, in which dynamic trajectories of moving objects are used to reconstruct fluorescence images of cytoskeletal structures. DOT microscopy takes time-lapse measurements of fluorescence images of moving organelles, single molecules or particles in live cells. The trajectory taken by the particle over time marks out the microtubule or actin filament that it travels along. At each time frame, particle positions can be determined with high accuracy by PSF fitting to a 2D Gaussian function to get a resolution that breaks the diffraction limit. We have applied this technique to track retrograde transportation of endosomes containing single NGF molecules in the axons of neurons. A pseudo Total Internal Reflection Fluorescence (TIRF) microscope was constructed in house to take the measurements. Individual NGF molecules conjugated to single quantum dots were imaged and tracked in live cells. The sub-diffraction-limit DOT imaging of individual axonal microtubules was achieved and we are able to clearly depict more than five microtubules in a single axon.

1922-Plat
Next Generation SICM Allows Nanoscale Imaging Of Biological Processes In Real-time
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The surface of a living cell is a complex three dimensional structure which is critical to the cell's function. Understanding the relationship between cell structure and function is essential to cell physiological research. Yet techniques for real-time imaging of the complex membrane cellular structures and investigation of the membrane functions of intact living cells are limited. Scanning ion conductance microscopy (SICM) is a relatively new form of Scanning Probe Microscopy, which enables non-contact high resolution imaging of living cells under physiological conditions using a fine nanopipette. Here we present major developments in SICM that will allow imaging of even the most complex structures and processes on live cells under physiological and pathophysiological conditions. Our new method is based on changing the way the pipette moves over the surface which now enables us to image the topography of highly complex live samples, such as neuronal networks, with a resolution better than 20 nm. We then go on to show that we can use this method to directly image the changes in cell surface topography during clathrin-mediated endocytosis in real-time.

1923-Plat
Optical Lock-in Detection (OLID) and OLID-FRET Imaging Microscopy
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The success of imaging fluorescent proteins within living cells is limited by the presence of background signals - while some improvements to image contrast can be achieved through background subtraction, these benefits diminish when the background signal varies in time and space. Further this approach does not allow imaging below the background signal, a regime of interest for single molecule biology. We have developed an optical lock-in detection (OLID) approach and associated signal analysis that involve modulating the fluorescence emission of the probe through deterministic, optical control of its fluorescent and non-fluorescent states, and subsequently applying a lock-in detection method to isolate the modulated signal of interest from non-modulated background signals. The lock-in detection method involves pixel-by-pixel based cross-correlation analysis that maps the correlation between the total fluorescence emission within single pixels of an image detected over several cycles of optical switching, and a reference waveform that represents the defined response of the switch probe to the switching cycle. The fundamentally new approach to imaging, which is similar in principle to radar, allows for the selective detection of emission from optical switch probes even in the presence of a larger population of conventional fluorescent probes. We have developed new synthetic and genetically-encoded optical switches for OLID and OLID-FRET imaging in living cells and tissues including nitrospirobenzopyran and naphthozaxine based probes as well as fusion proteins of Dronpa and a new optically switchable mcherry. OLID imaging using these probes is remarkably efficient at imaging specific structures and proteins in high and time varying background signal environments such as in living cells, in culture, in brain slices and in live *Xenopus* embryos and zebrafish larvae.

1924-Plat
Multiparameter Spectroscopy For Single-Molecule Fluorescence And Confocal Imaging
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Multiparameter fluorescence detection (MFD) is a technique that simultaneously records all information observable in a fluorescence experiment, i.e. polarization, color, photon arrival time and spatial origin. This is particularly useful in single molecule studies, where sequential measurements of different properties could not easily account for temporal variations in heterogeneous or unstable samples, but also in low light level investigations where the number of available photons is very limited. In MFD a single raw data set delivers fluorescence decay histograms, anisotropy decay histograms, intensity ratios and time traces or fluorescence correlation curves for individual molecules or sub-ensembles with spatial resolution close to the diffraction limit. The technique requires single photon sensitive detectors, high speed data acquisition and sophisticated software to analyze the data. In a confocal environment imaging can be achieved either by beam scanning or by stage scanning, depending on the requirement of the experiment.
A general overview, a study of rotational Brownian motion of biomolecules by high-resolution fluorescence correlation spectroscopy (FCS) and some applications in confocal imaging will be presented.

1925-Plat
Dual Objective Multifocal Plane Microscopy for Single Particle/Molecule Imaging Applications
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In single molecule/particle imaging applications, the number of photons detected from the fluorescent label plays a crucial role in the quantitative analysis of the acquired data. For example, in 2D/3D tracking experiments the localization accuracy of the labeled entity is inversely proportional to the square root of the number of detected photons. Hence achieving high photon collection efficiency is important in such studies. Currently, single molecule/particle imaging experiments are typically carried out on either an inverted or an upright microscope, in which the photons are collected from only one side of the sample (i.e., top side or the bottom side).
Here, we report the development of dual objective multifocal plane microscopy (dMUM) for single particle/molecule studies. The dMUM configuration uses two opposing objective lenses, where one of the objectives is in an inverted position and the other objective is in an upright position. We show that dMUM has higher photon collection efficiency than a regular (inverted/upright) microscope for a given illumination condition. Because of the presence of two objective lenses, dMUM supports simultaneous imaging of different focal planes. This has been shown to be beneficial for high accuracy 3D localization and tracking of single molecules/particles and sub-cellular objects over a wide spatial range in live cells [1-3]. We demonstrate that fluorescent labels can be localized in 2D/3D with better accuracy when imaged through dMUM than when imaged through a regular (inverted/upright) microscope configuration. Analytical tools are introduced to estimate the 2D/3D location from dMUM images and to characterize the accuracy with which they can be determined.
1. Prabhat, P. et al., IEEE Trans. Nanobiosci., 2004, 3, 237-242.
2. Prabhat, P. et al., Proc. Natl. Acad. Sci., USA, 2007, 6443, D1-D7.
3. Ram S., et al., Biophys. J., 2008, doi:10.1529/biophysj.108.1403